Development of Integrase Inhibitors of Quinolone Acid Derivatives for Treatment of AIDS: An Overview

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Abstract: HIV-1 integrase (IN), which has no cellular counterpart, has been intensely studied over the past 15 years and has been fully validated as a therapeutic target with the first FDA approved IN inhibitor raltegravir. The quinolone acid GS-9137 (elvitegravir), which most probably will become the next candidate of IN inhibitors, is in the process of enrolling patients in the phase III clinical trials. This review focuses on small-molecular of quinolone acid derivatives, which have the similar pharmacophore of *β*-diketoacids, as integrase inhibitors with antiviral activity.

Keywords: Drug discovery, diketoacides, HIV-1, inhibitors, integrase, quinolone acid derivatives.

INTRODUCTION

Since the first case of HIV/AIDS was identified, AIDS has become the largest and most devastating public health pandemic. An estimated 2.7 million new HIV infections occurred and 2 million died due to AIDS-related illnesses worldwide in 2008 [1]. Recent failures in efforts to develop an effective vaccine against HIV-1 infection have emphasized the importance of antiretroviral therapy in treating HIV-1 infected patients [2]. Thus far, inhibitors of two viral enzymes, reverse transcriptase (RT) and protease have had a profoundly positive impact on the survival of HIV-1-infected patients. Therefore, new inhibitors that act at diverse steps in the viral replication cycle are urgently needed because of the development of resistance to currently available antiretroviral drugs [3].

Within 25 years after zidovudine was first discovered as an inhibitor of HIV replication, 25 anti-HIV drugs have been formally approved by the US Food and Drug Administration (FDA) for clinical use in the treatment of HIV infections: eight nucleoside reverse transcriptase inhibitors (NRTIs); four non-nucleoside reverse transcriptase inhibitors (NNRTIs); ten protease inhibitors (PIs); one fusion inhibitor (FI); one co-receptor inhibitor (CRI) and one integrase inhibitor (INI). These compounds were used in various drug combination (some at fixed dose) regimens so as to achieve the highest possible benefit and tolerability, and to diminish the risk of virus-drug resistance development. The highly active antiretroviral therapy (HAART) combination regimens suppressed viral loads to undetectable levels but failed to eradicate the HIV-1 virus from infected patients. Because of the chronic nature of HIV-1 infection, a long HAART regimen is required for infected patients. Rapidly emerging multidrug resistant HIV-1 virus strains and severe adverse effects from long-term HAART medication necessitated all kinds of potent and safe drugs targeting alternative steps in the HIV-1 replication process [4-8].

HIV-1 integrase is an essential enzyme for retroviral replication, it is involved in the integration of HIV DNA into host chromosomal DNA. There appears to be no functional equivalent in human cells and the reactions catalyzed by IN are unique [9]. The recent progress in the discovery and clinical development of HIV-1 integrase inhibitors as novel antiretroviral agents stimulates a new hope in the treatment of AIDS [10-15] patients.

THE STRUCTURE AND FUNCTION OF HIV-1 INTEGRASE

The Structure of HIV-1 Integrase

The three viral enzymes (protease, reverse transcriptase and integrase) are encoded within the HIV *Pol* gene and translated as a polyprotein. IN is released from the polyprotein by protease cleavage during maturation. IN is a 32-kDa protein that comprises three domains [16-18]: the amino-terminal domain (NTD) (residues 1-50), which contains a conserved HHCC-binding motif that coordinates one zinc atom (Fig. (**1**)); the catalytic core domain (CCD) (residues 51-211), which contains a canonical three-amino acid DDE motif corresponding to D64, D116 and E152; The carboxyterminal domain (CTD) (residues 212-288) is the least conserved among retroviral integrases but has an overall SH3 fold [16]. Mutation of any of these residues in CCD region abolishes IN enzymatic activities and viral replication [16-18]; and these residues in CCD region coordinate presumably two divalent metal ions (Mg^{2+} or Mn^{2+}) in complex with the viral and host DNA [16-18]. The CTD binds to a broad range of DNA sequences. A few research in the field suggested that IN might exist as a tetramer in human cells

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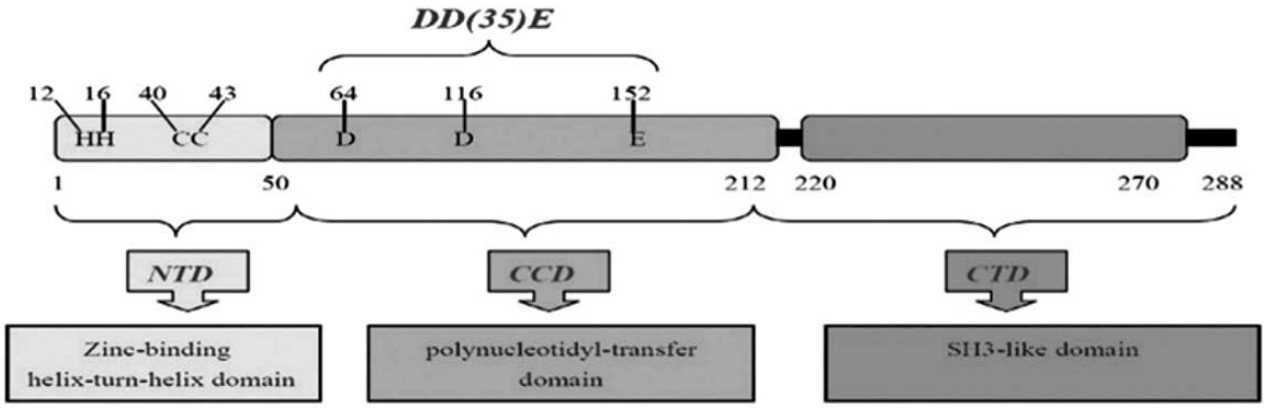


Fig. (1). Schematic diagram of the HIV-1 integrase three domains including the N-terminal domain (NTD), the catalytic core domain (CCD) and the C-terminal domain (CTD).

but the arrangement of IN plus viral and host DNA has not yet been elucidated by crystallography [16].

Mechanism of Integration

IN mediates the integration of the viral cDNA into the host genome through two successive reactions (Fig. (**2a**, **2b**, **2c**) [19-22]. In the first reaction, which takes place in the cytoplasm of an infected cell, IN recognizes newly transcribed viral cDNA and specifically cleaves two nucleotides (GT) from its highly conserved CAGT-3′ ends and produces nucleophilic hydroxyls on both 3′-ends (3′-CA-OH) of the viral cDNA. This reaction is termed 3′-processing (3'-P). Subsequently, IN is transported into the nucleus of the host cell along with the processed viral cDNA and several cellular cofactors as a part of the reintegration complex. In the second reaction, which takes place in the nucleus of the infected cell, IN mediates the nucleophilic attack of both 3′-end hydroxyls of the viral cDNA onto the host DNA. This insertion (integration) process is termed strand transfer (ST). ST consists of ligation of the two 3'-hydroxyl ends into the host chromosome with a five base-pair stagger across the DNA major groove [20]. This reaction results in a two-base overhang on the 5'-end of the viral cDNA and a five-base single-stranded gap at each junction. The trimming of viral 5'-ends and gap repair of the duplex DNA structure is probably completed by the DNA repair enzymes from the host cell, although RT has been proposed to be involved in this reaction [20].

THE MECHANISM TO INHIBIT VIRAL INTEGRATION OF DIKETO-ACID DERIVATIVES

The most successful class of IN inhibitors, the diketo-acid derivatives, selectively inhibits the ST reaction. Diketo acids might bind at the interface of the IN–DNA–divalent metal complex to the acceptor site (Fig. (**2d**, **2e**) [19, 20, 23]. The mechanism of selective ST inhibition had been elucidated by several important experiments. The first significant test was obtained by a crystal structure of the inhibitor 5-CITEP in complex with the catalytic core of IN [24]. In this structure, the inhibitor was bound in the center of the catalytic active site, lying between the three residues of the DDE motif without displacing the metal ions. The next key experiment was that inhibitor binding required a catalytically active IN assembled on viral DNA ends. Target (host chromosomal) DNA substrates, that are competent for ST, were effective competitors of inhibitor binding, suggesting that the host DNA and inhibitor occupy similar sites in the IN complex [25, 26]. Further mechanistic insights were provided by experiments establishing that inhibitor binding and function were dependent on the metal-ion cofactors coordinated with the DDE motif [26, 27]. Researchers proposed that a two-metal binding model as a potential mechanism of chelating inhibitors against HIV IN, potential inhibitors would bind to two metal ions (Mg^{2+} and Mn^{2+}) in the active site of HIV IN to prevent human DNA from undergoing the integration reaction [28]. Based on these key experiment data, interfacial inhibition had been hypothesized as a mechanism of action of selective ST inhibitors [20, 27]. In this model it was proposed that the ST inhibitors preferentially bind a structural intermediate at the interface of the viral DNA-IN–divalent metal complex formed during the 3'-P step. Upon inhibitor binding, the 3'-P intermediate was stabilized, IN is unable to bind target chromosomal DNA and the ST reaction is unable to proceed [20]. *In vitro* studies revealed that the majority of resistance-conferring mutations clustered around the IN catalytic active site (the DDE motif) [29-33]. That made sure the mechanism of action of ST transfer inhibitors.

AN OVERVIEW OF INTEGRASE INHIBITORS

While many structurally diverse compounds have been reported to be inhibitors of HIV integrase, only a few compounds of one kind, the β-diketoacids, its isosteres and related compounds represent the most convincing, biologically validated inhibitors of this viral enzyme [18, 34, 35]. Over the past 15 years, a variety of IN inhibitors containing β-diketoacid moiety had been discovered. Compound **1** (L-708,906) [29], was one of the earliest examples of an aromatic diketo acid with HIV-1 integrase inhibitory activity. Then the aromatic diketo acids were typically replaced by: 1) heterocyclic diketo acids, such as compounds **2** (L-731-988) [36], **3** [37], **4** [38], **5** (S-1360) [35] and **6** (5-CITEP) [9]; 2) bifunctional diketo acids, such as compound **7** [9]; 3) naphthyridine carboxamides, such as compounds **8** (L-870,810) [30] and **9** (L-870,812) [39]; 4) tricyclic phthalimides, such as compound **10** [40]; 5) dihydroxypyrimidine carboxamides, such as compounds **11** [41] and **12** (MK-0518) [42]; 6) quinolinone acids, such as compound **13** (GS-9137) [43].

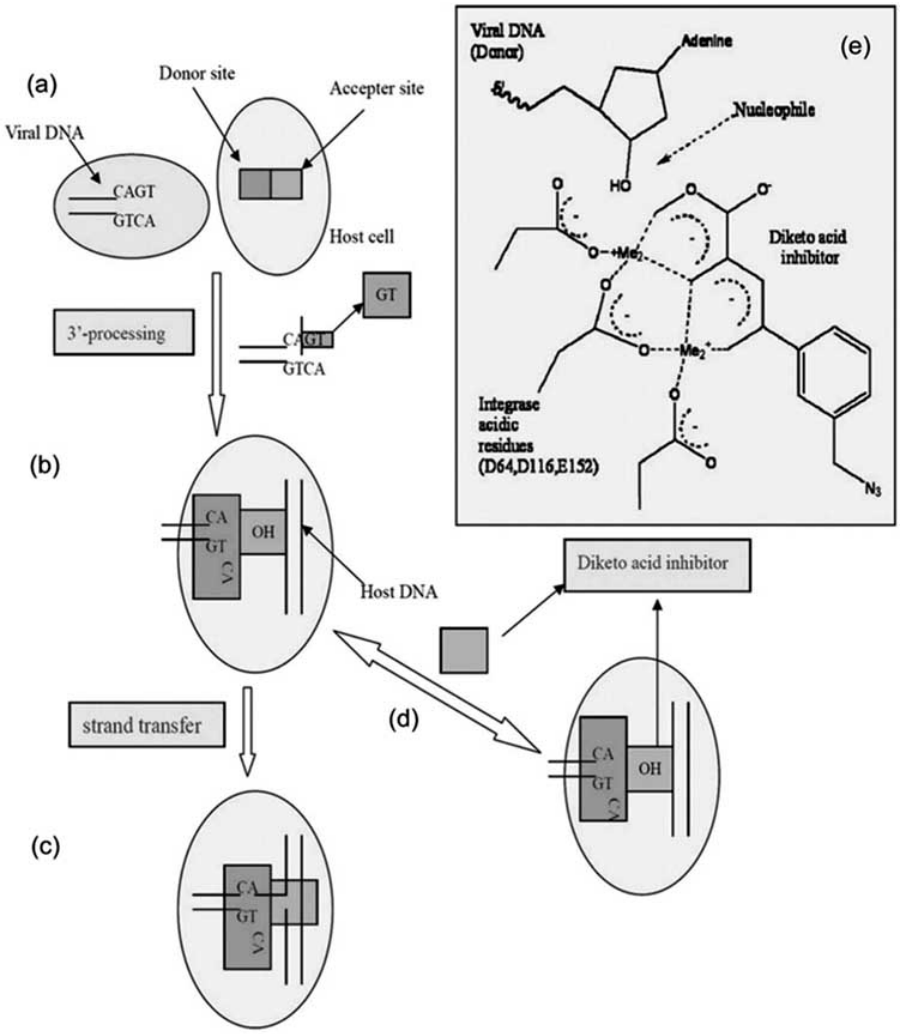
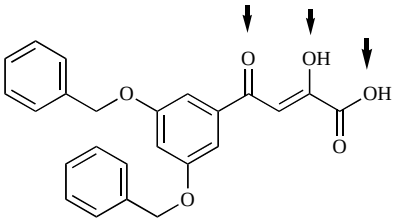


Fig. (2). Schematic diagram of integrase-mediated 3'-processing and strand transfer reactions and inhibition by diketo acid inhibitor.

S-1360 was the first IN inhibitor to enter clinical trials, but unfortunately failed efficacy tests due to the metabolism and clearance of the molecule *via* a non-cytochrome P450 clearance pathway. It was later discovered that S-1360 was a substrate for the aldo-keto reductase family of enzymes, which are NADPH-dependent, carbonyl reducing enzymes in the human liver [44]. Pharmacokinetic data from phase II clinical trials showed very low plasma concentrations of S-1360 in a majority of subjects, which, at least in part, may be due to the rapid reduction of S-1360 followed by glucuronidation. The development of S-1360 by Shionogi & Co. and GlaxoSmithKline had been discontinued. Later studies at Merck Laboratories resulted in the discovery of the naphthyridine carboxamide class with diketo pharmacophore of IN inhibitors. Clinical evaluation on two candidates, L-870,810 and L-870,812, had been undertaken. Unfortunately following initial success in L-870,810 short-term monotherapy in both naive and treatment experienced HIV-1 infected patients, the clinical trials of this inhibitor was halted due to an observed long-term dosing toxicity in the liver and kidneys of dogs [45]. Additionally L-870,810 exhibits a high affinity for serum protein binding, which may result in a lower effective plasma drug concentration. The clinical status of L-870,812 had not been made public, but considering the structural similarity to L-870,810 it was likely to display similar *in vivo* characteristics. MK-0518, a pyrimidinone carboxamide, had displayed favorable results in phase I, II, and III studies, and received FDA approval as the first IN drugs for treatment AIDS in October 2007. GS-9137, a quinolone carboxylic acid, had exhibited favorable results in both phase I and II studies, and was undergoing phase III trials [46].

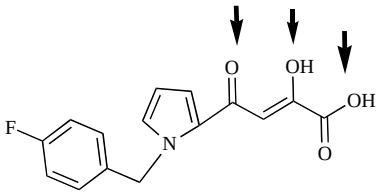
DEVELOPMENT OF QUINOLONE DERIVATIVES AS INTEGRASE INHIBITORS

Quinolone derivatives are small extremely versatile molecules, easily synthesized at low cost on a large scale and endowed with well-known biochemical properties that make them very suitable pharmacophore structures. They originally were synthesized as potential antibacterial [47, 48]. Researchers [49] found compound **14** exhibited anti-HIV activities against HIV-1_{IIIB} chronically infected MT-4 cells, with IC_{50} = 1.7 μM and CC_{50} = 60 μM. When the bridge moiety of the core skeleton of **14** between the N-1 and C-8 position was cut off and substituted with a cyclopropyl group at N-1 position, compound **15** (IC_{50} = 0.059 μM and CC_{50} = 5.7 μM) was more potent than **14**.



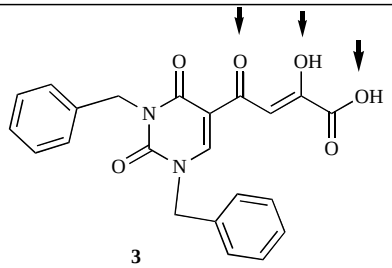
1 (L-708, 906)

(ST, IC_{50} = 0.1 mM, EC50 < 2 mM)



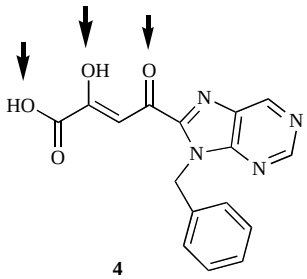
2 (L-731, 988)

(ST, IC_{50} = 0.17 μM, EC_{50} = 9.6 μM)



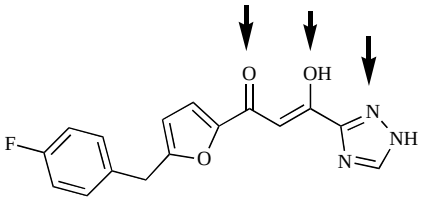
3

(ST, IC_{50} = 0.2 μM, EC_{50} = 1.54 μM)



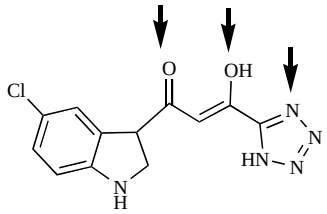
4

(ST, IC_{50} = 10 μM)



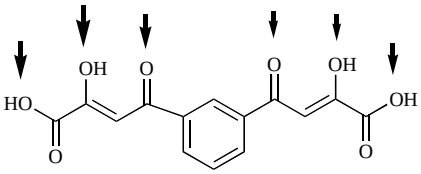
5 (S-1360)

(ST, IC_{50} = 0.02 μM, EC_{50} = 0.14 μM)



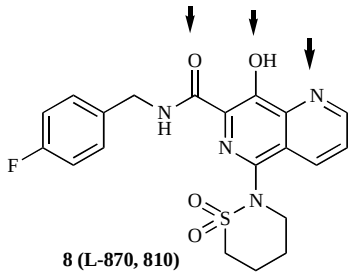
6 (5-CITEP)

(ST, IC_{50} = 0.65 μM)



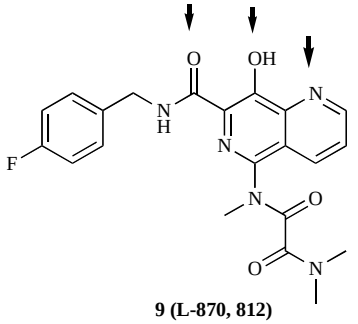
7

(ST, IC_{50} = 1.83 μM)



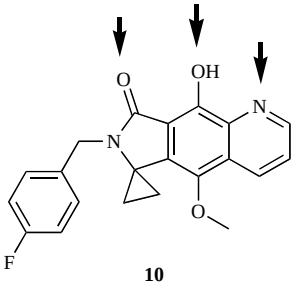
8 (L-870, 810)

(ST, IC_{50} = 15 nM, EC_{50} = 3.6 nM)



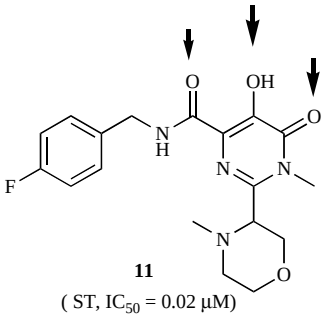
9 (L-870, 812)

(ST, IC_{50} = 40 nM, EC_{50} = 250 nM)



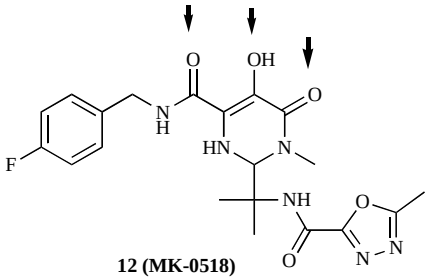
10

(ST, IC_{50} = 0.007 μM)



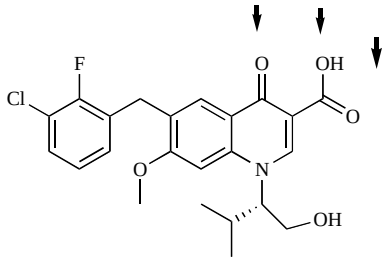
11

(ST, IC_{50} = 0.02 μM)



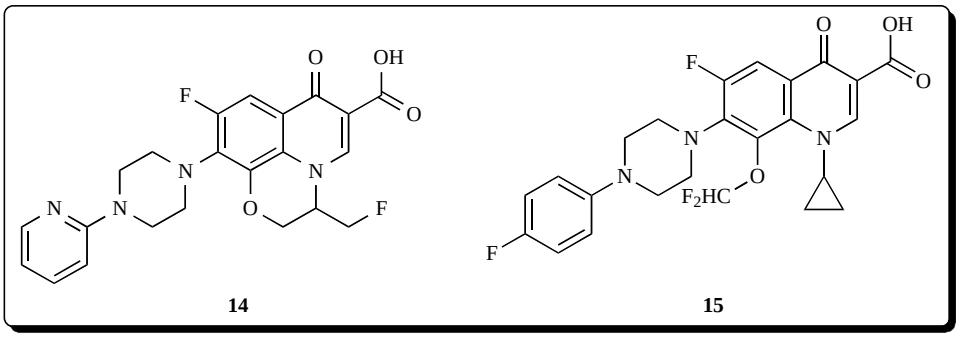
12 (MK-0518)

(ST, IC_{50} = 27 nM, EC_{50} = 6 nM)



13 (GS-9137, JTK-303)

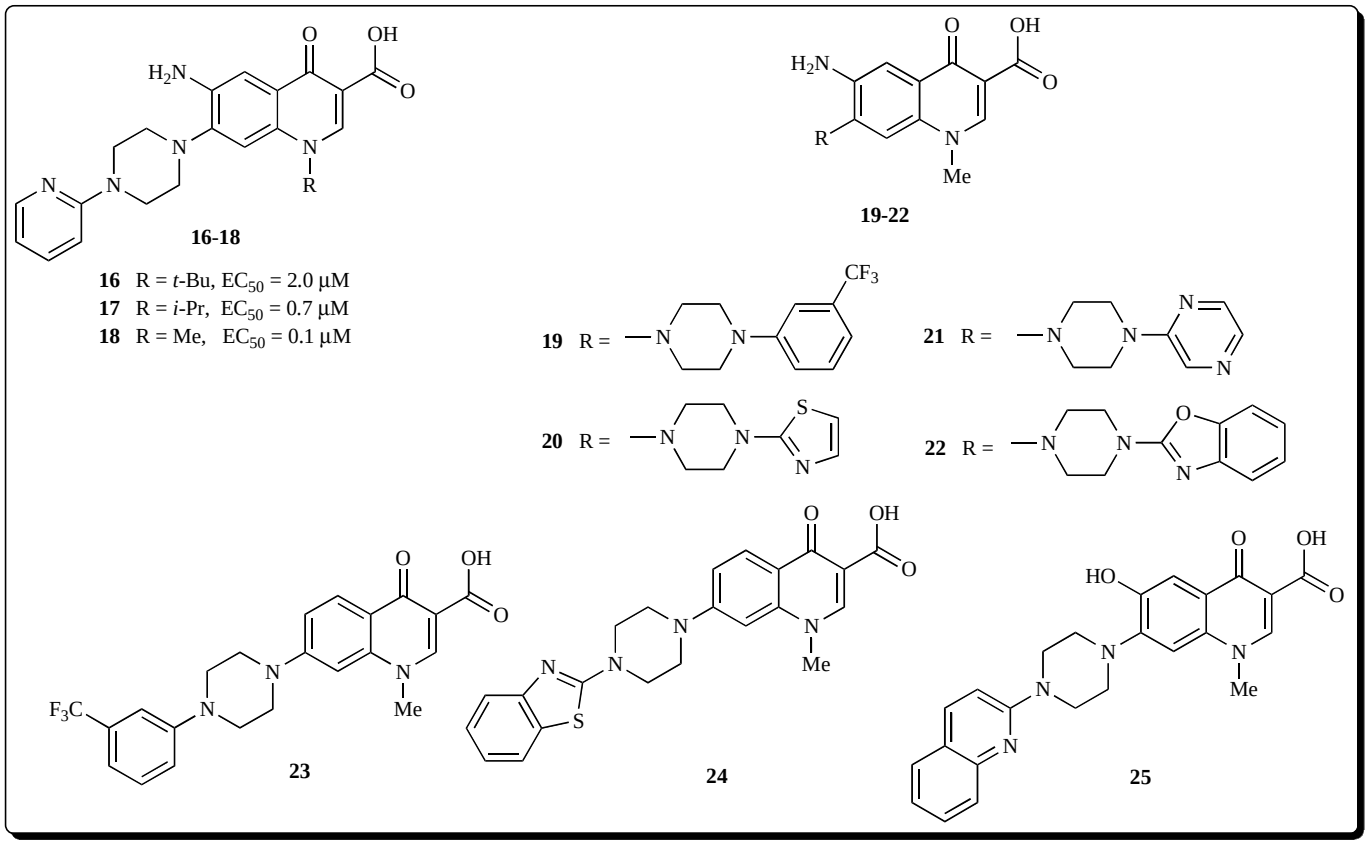
(ST, IC_{50} = 7.2 nM, EC_{50} = 0.9 nM)



14 **15**

When the fluorine at C-6 position of quinolone was replaced by amino group, the compounds **16-18** also exhibited anti-HIV activities [50]. The most potent inhibitor in those molecules was compound **18**, which bears a methyl group at the N-1 position. The results of 3D-QSAR study [51] confirmed that high antiviral activity could be ensured by the hydrophilic region around the 4-keto-3-carboxylic moiety and a suitable hydrophobic region around the C-7 position. Moreover high antiviral activity seems to be related the presence of a hydrophobic small substituent at N-1 position. For the further structure-activity relationship (SAR) study of 6-aminoquinolone, compound **18** was as a lead compound and modified at C-7 position by replacing the pyridine ring at the N-4 piperazine core with aromatic heterocyclic or benzoheterocyclic groups such as compounds **19-22** [52]. The C-7 thiazolpiperazinyl derivative **20** (EC_{50} = 0.13 μg/mL, CC_{50} = 2.18 μg/mL) and C-7 benzoxazolpiperazinyl derivative **22** (EC_{50} = 0.015 μg/mL, CC_{50} = 0.0098 μg/mL) proved to be more potent than compound **18** (EC_{50} = 0.29 μg/mL, CC_{50} = 0.71 μg/mL) inhibiting the replication of HIV-1_{IIIB} in MT-4 cells. Compound **19** (EC_{50} = 0.31 μg/mL, CC_{50} = 2.18 μg/mL) and pyrazinylpiperazine derivative **21** (EC_{50} = 0.70 μg/mL, CC_{50} = 3.98 μg/mL) also showed nearly as good antiviral activity as compound **18**. The increased antiviral activity was also coupled with a markedly lower cytotoxicity in CEM cells. In particular, derivatives **20**, **21** as well as the lead **18**, were devoid of any cytotoxicity (CC_{50} >100 μg/mL). While on PBMCs the compounds **18-22** showed a cytotoxicity that was on the same order as that observed on MT-4 cells.

When the amino group were replaced by hydrogen or hydroxyl at C-6 position, compound **23** (EC_{50} = 0.07 μg/mL, CC_{50} = 2.61 μg/mL), **24** (EC_{50} = 0.03 μg/mL, CC_{50} = 0.44 μg/mL) and **25** (EC_{50} = 0.08 μg/mL, CC_{50} = 0.47 μg/mL) exhibited good antiviral activity inhibiting the repli-



16-18

16 R = *t*-Bu, EC_{50} = 2.0 μM
17 R = *i*-Pr, EC_{50} = 0.7 μM
18 R = Me, EC_{50} = 0.1 μM

19-22

19 R =
21 R =
20 R =
22 R =

23 **24** **25**

cation of HIV-1_{IIIB} in MT-4 cells [53, 54]. While the amino group was replaced by trifluoromethyl and methoxyl, all of these derivatives were especially devoid of any antiviral properties at concentrations below the cytotoxic levels.

Some bifunctional *β*-diketo acids (such as compound **7**) were less potent than mono functional counterparts and endowed with low antiviral activities. These properties could be ascribed to the lack of some important structural features. In fact, structure-activity relationships on monofunctional aryl diketo acids led to the conclusion that the highest activity was obtained when the central aromatic ring was 1,3-disubstituted with the diketo acid chain and a benzyl moiety [37]. In such a way, the angle between the two lines extended from the above groups was around 120° [37]. According to this research result, Di Santo *et al*. [55] designed the bifunctional *β*-diketo acids scaffold, the 4-(4(1*H*)-quinolinon-3-yl)-2,4-dioxobutanoic acid skeleton (Fig. (**3**)). They chose the scaffold because it is easy to alkylate the 4(1*H*)-quinolinone at N-1 position with a benzyl group to obtain a 1,3-disubstituted compound that well fits the geometric requirements for an optimal IN inhibitory activity and a second diketo acid function can readily be introduced at position 6 of the aromatic ring *via* an acetyl intermediate. Based on the scaffold, researchers designed a series of new inhibitors **26**-**29** [55], which exhibited potent inhibitory activity against IN for both ST and 3'-P steps. The acid derivatives (**27**, **29**) were more potent than the corresponding esters derivatives (**26**, **28**), and the *p*-F-benzyl-substituted compounds (**28**, **29**) at N-1 position were more active than the unsubstituted counterparts (**26**, **27**). Compound **29** (ST, IC_{50} = 0.015 μM, 3'-P, IC_{50} = 0.44 μM; CC_{50} > 200 μM, EC_{50} = 4.29 μM) was the most potent inhibitor with IC_{50} values for strand transfer around 15 nM. Compound **29** was selective for ST inhibition with IC_{50} values approximately 30-fold lower for ST than for 3'-P.

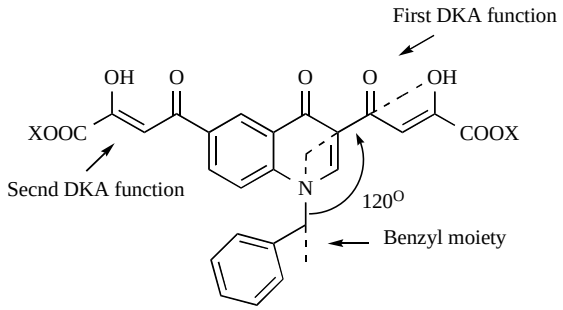
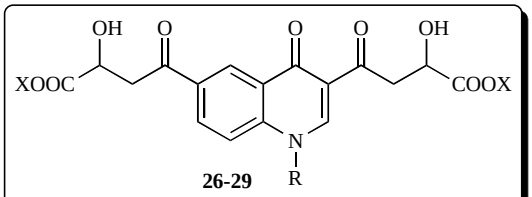


Fig. (3). Structural features of the newly designed bifunctional quinolonyl diketo acid derivatives [55].



26-29

26	R = H	X = Et
27	R = H	X = H
28	R = 4-F-Ph-CH_2-	X = Et
29	R = 4-F-Ph-CH_2-	X = H

Based on the docking research result, two predominant binding mode of the most active compound **29** within the IN catalytic core domain (CCD) were supposed (Fig. (**4**)). In Fig. (**4a**), the carboxylate group of one diketo acid chain of the ligand chelates the Mg^{2+}, whereas the other one inserts between residues K156 and K159, formed hydrogen bonds with both side chains and with N155 CO backbone. The *p*-F-benzyl group pointed toward a hydrophobic pocket formed by the catalytic loop residues Y143, P142, I141, and G140 and by residues I60, Q62, V77, V79, H114, G149, V150, I151, E152, S153, and M154. Particularly, a favorable electrostatic interaction occurred between the fluorine atom on the benzyl ring and the amide group of Q62 residue. The quinoline ring of the molecule formed a stacked amide-aromatic interaction with the N155 side chain, while the quinoline carbonyl oxygen enabled a hydrogen bond to the T66 side chain. In Fig. (**4b**), the metal ion was chelated in a bidentate manner by the quinoline carbonyl group and by oxygen of the diketo acid function, while the hydroxyl and the carboxylate groups of the same branch contacted the D116 and the N117 side chains, respectively. On the opposite side of the molecule, the other diketo acid arm elongated toward the K156 and K159 residues contacting exclusively the latter amino acid. The *p*-F-benzyl group entirely was inserted in the hydrophobic pocket, where it was stabilized by hydrophobic interactions with I151 and P142 side chains. Although the two binding modes differed in some details, it was worth noting that, in both docking results, the ligand interacted with the same enzyme attachment points: (i) the metal ion, (ii) the K156 and/or K159 residues, and (iii) the hydrophobic pocket.

The novel quinoloneyl diketo acid derivatives, which were designed by replacement of the 6-diketo acid chain of **29** with smaller substituent in the 6-, 7-, or 8-position of the quinolinone ring, exhibited high potency against IN with high selectivity against ST [56, 57], such as compound **30** (ST, IC_{50} = 0.018 μM, CC_{50} > 200 μM, EC_{50} > 50 μM) and compound **31** (ST, IC_{50} = 0.03 μM, 3'-P, IC_{50} = 1.6 μM, CC_{50} > 1000 μM, EC_{50} = 4.0 μM).

Sato *et al*. reported the novel IN inhibitor GS-9137 (**13**), that blocks strand transfer by viral enzyme [43]. The coplanar monoketo acid motif in 4-quinolone-3-carboxylic acid **32** could be an alternative to the diketo acid motif and provided novel insight into the structural requirements and the binding mode of this type of inhibitors. Quinolone derivatives **32** had an IC_{50} of 1.6 μM in the strand transfer assay. Introduction of 2-fluoro and 3-chloro substituent into the distal benzene ring of compound **32**, compound **33** had a significant improvement inhibition of strand transfer (IC_{50} = 44 nM) and of antiviral activity (EC_{50} = 0.81 μM). Compound **34**, bearing a hydroxyethyl group at the N-1 position of the quinolone ring, was 1.8-fold more potent at inhibiting strand transfer (IC_{50} = 24 nM) and displayed about 11-fold stronger antiviral activity (EC_{50} = 76 nM) than compound **33**. Introduction of a methoxy group at the C-7 position of the quinolone ring of **34**, compound **35** had a significant improvement of inhibition of strand transfer (IC_{50} = 9.1 nM) and of antiviral activity (EC_{50} = 17 nM). Compound **36**, bearing an isopropyl group at the 1*S*-position of the hydroxyethyl moiety, was about 3-fold more potent at inhibiting strand transfer

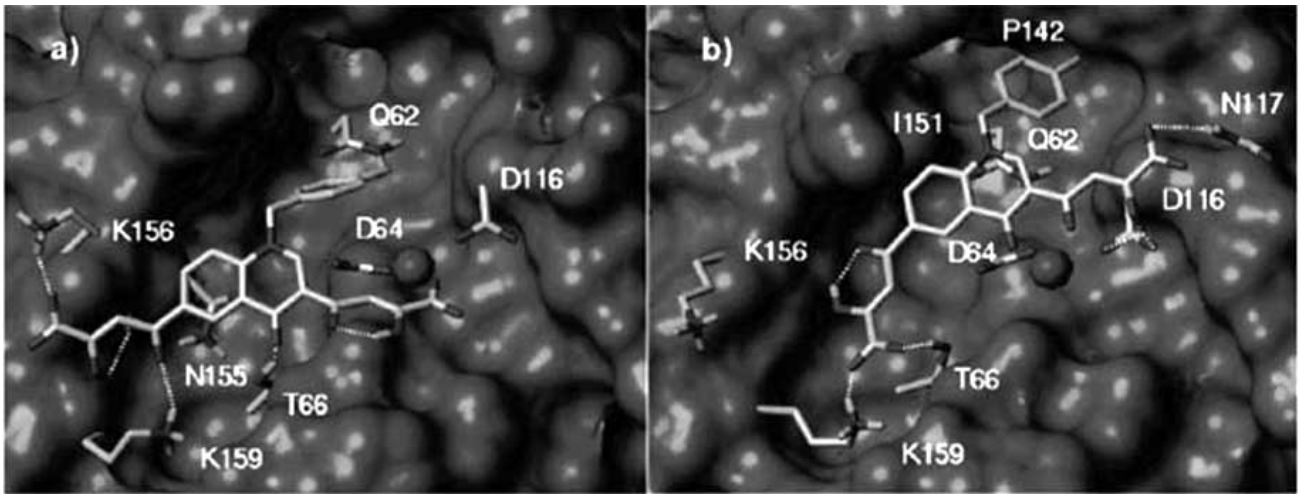
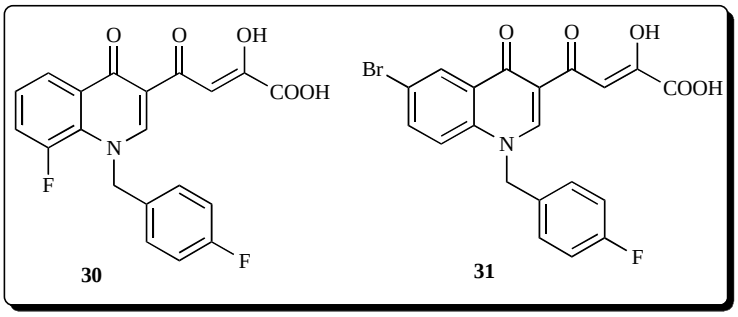
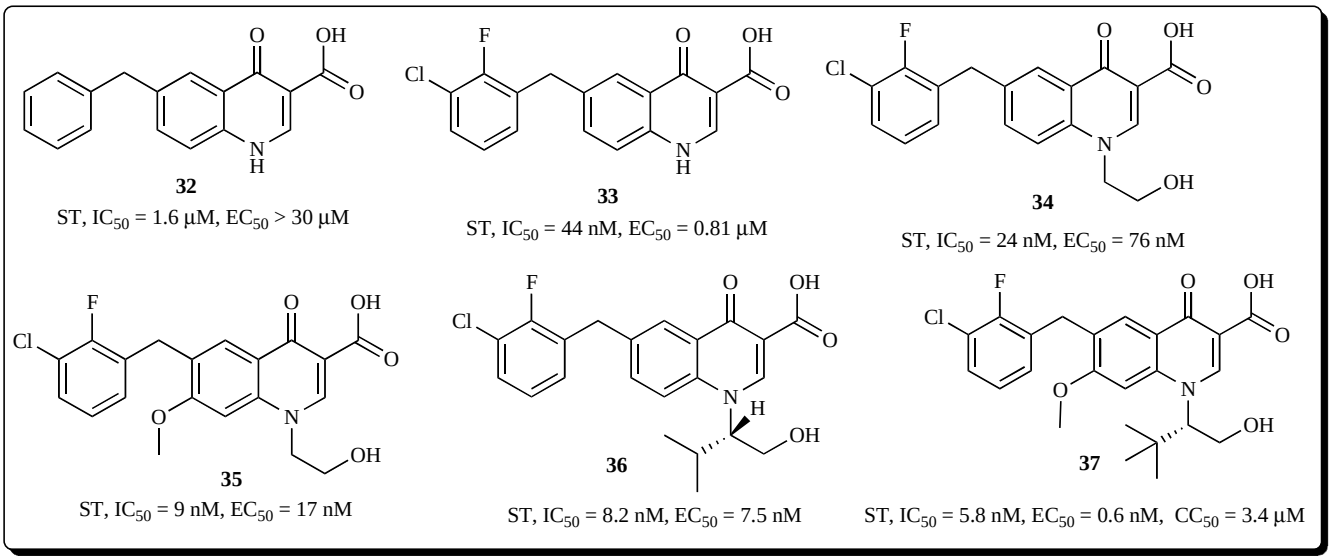


Fig. (4). The two predominant binding modes of **29** to MD snapshots of the IN core domain. Residues lining the ligand position are highlighted. The metal ion is represented as a sphere [55].



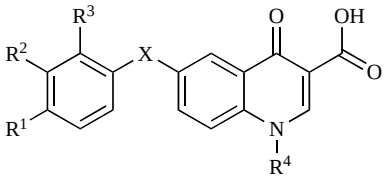
(IC_{50} = 8.2 nM) and about 10-fold stronger at inhibiting HIV replication (EC_{50} = 7.5 nM) than **34**. Introduction of both a methoxy group at the C-7 position of the quinolone ring and an isopropyl group at the 1*S*-position of the hydroxyethyl moiety of **34**, compound **13** had a synergistic improvement of antiviral activity (EC_{50} = 0.9 nM), but there was no additive or synergistic improvement in the inhibition of HIV-1 integrase (IC_{50} = 7.2nM). In 2009, Sato *et al*. presented more detailed information on the SAR of the novel class of quinolone integrase inhibitors and their effects on strand transfer reaction, 3'-processing and viral replication [58]. They designed all kinds of the quinolone derivatives with different substituent at N-1, C-3, C-6 and C-7 position, which showed different potent inhibition activity of ST, 3'-processing and viral replication. They found the independent introduction of a small alkyl group or a hydroxyethyl group at the N-1 posi-



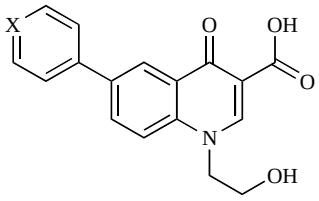
tion of the quinolone ring led to a significant improvement of activity. They introduced several alkyl groups, such as a methyl, an ethyl, a n-propyl, an isopropyl, a tert-butyl, or a cyclohexyl group, at the 1S-position of the hydroxyethyl moiety. Among these designed molecules, compound **37** (ST，IC_{50} = 5.8 nM，EC_{50} = 0.6 nM，CC_{50} = 3.4 μM) displayed the more potent inhibition activity of ST and antiviral activity than **13**.

Based on the IN inhibitor GS-9137 (**13**), researchers of Belgium retained the pharmacophoric groups of GS-9137 and decorated at N-1 and C-6 to acquire the compounds **38-57** [59]. Hydroxyalkyl chains of variable length were introduced at N-1 to study the effect of the spacer between the heterocyclic nitrogen and the OH group. Moreover, chains with a terminal alkoxy moiety or basic nitrogen were used to evaluate the importance of a free OH and the influence of a protonatable group on the anti-IN activity of the molecule. In most of the compounds, the benzyl group of GS-9137 was replaced with potential bioisosteric moieties with the aim of exploring novel structural motifs and establishing structure-activity relationships. No one of the new compounds a methoxy group at C-7 was introduced because this substituent, although important for pharmacokinetic and *in vivo* activity profiles, did not affect *in vitro* binding affinity, as demonstrated by the comparable IC_{50} values of **36** and its 7-demethoxy derivative **13** (7.2 nM vs. 8.1 nM, respectively). When the methylene at C-6 position was replaced by oxygen, compound **38** proved to be completely inactive in the ST assay (IC_{50} > 100 μM), and still had some extent antiviral activity inhibiting the replication of HIV-1_{IIIB} in MT-4 cells (EC_{50} > 9.9 μM). While the inhibitory potency was partially restored with a thiophenyl group even if unsubstituted (**39**, IC_{50} = 18.5 μM). Compounds **40-42**, characterized by a hydroxyalkyl chain of variable length at N-1 and a 2,4-dichlorobenzyl moiety at C-6, showed anti-IN activity in the low micromolar range, which was at least 20 times less potent than the compound **13** in the ST assay. The introduction of a methoxyethyl or a dimethylaminoethyl chain at N-1 resulted in **43** and **44**, respectively, which a further significant decreased in activity. When the methylene at C-6 position was replaced by carbonyl, ethylene, amide, inverse amide, and amino group, compounds **45-52** proved to be completely inactive (IC_{50} > 100 μM) or a loss activity in the ST assay. The docking study suggested an electron withdrawing effect on the chelating system due to the C-6 carbonyl substituent, which resulted in a poor interaction of the molecules with the divalent metal ions of the active site. Removal of the spacer between the two aromatic rings gave **53** and **54**, characterized by a phenyl or a pyridyl group directly linked to C-6, which proved to be completely inactive in inhibiting the enzyme. The most interesting thing was that the compounds **55** (IC_{50} >100 μM), **56** (IC_{50} = 2.1 μM), and **57** (IC_{50} = 1.6 μM) with similar structure had different inhibition activity in the ST assay.

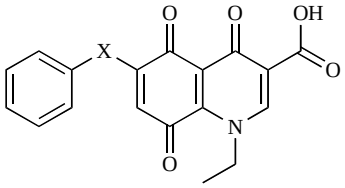
Designing multiple ligands had been a subject of growing interest in medicinal chemistry. Compounds active against both RT and IN were reported [60], though their usability was limited by toxicity and the lack of a general design strategy. Researchers designed compounds **58-61** with dually active, conjugates combining quinolone pharmacophore with pyrimidine for RT/IN dual inhibitors [61]. The assay results for compounds **58-61** demonstrated activity against RT at low to sub-micromolar range, while moderate activity against IN was observed, which validates quinolone carboxylic acid as a pharmacophore choice in designing RT/IN dual inhibitors. The anti-HIV activity from cell-based assay fell into the same range as the anti-RT activity, implying that the contribution of IN activity to the overall activity might not be significant.



38-52

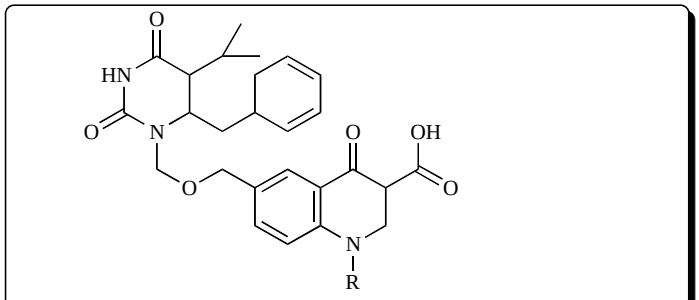


53 X = CH
54 X = N



55 X = NH, R^1 = 2-F, 3-Cl
56 X = NH, R^1 = 4-F, 3-Cl
57 X = S, R^1 = H

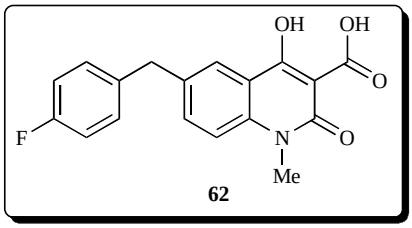
38 X = O, R^1 = F, R^2 = Cl, R^3 = H, R^4 = $(CH_2)_2OH$
39 X = S, R^1 = R^2 = R^3 = H, R^4 = $(CH_2)_2OH$
40 X = CH_2, R^1 = R^3 = Cl, R^2 = H, R^4 = $(CH_2)_2OH$
41 X = CH_2, R^1 = R^3 = Cl, R^2 = H, R^4 = $(CH_2)_3OH$
42 X = CH_2, R^1 = R^3 = Cl, R^2 = H, R^4 = $(CH_2)_4OH$
43 X = CH_2, R^1 = R^3 = Cl, R^2 = H, R^4 = $(CH_2)_4N(CH_3)_2$
44 X = CH_2, R^1 = R^3 = Cl, R^2 = H, R^4 = $(CH_2)_4OCH_3$
45 X = CO, R^1 = R^3 = Cl, R^2 = H, R^4 = $(CH_2)_2OH$
46 X = CO, R^1 = R^3 = Cl, R^2 = H, R^4 = $(CH_2)_3OH$
47 X = CO, R^1 = R^3 = Cl, R^2 = H, R^4 = $(CH_2)_4OH$
48 X = CHCH, R^1 = R^2 = R^3 = H, R^4 = $(CH_2)_2OH$
49 X = CONH, R^1 = R^2 = R^3 = H, R^4 = $(CH_2)_2OH$
50 X = NHCO, R^1 = R^2 = R^3 = H, R^4 = $(CH_2)_2OH$
51 X = NH, R^1 = H, R^2 = Cl, R^3 = F, R^4 = $(CH_2)_2OH$
52 X = NH, R^1 = H, R^2 = Cl, R^3 = F, R^4 = CH_2CH_3



58-61

58 R = H, RT: IC_{50} = 0.19 μM, IN: IC_{50} = 35 μM, EC_{50} = 0.22 μM
59 R = Me, RT: IC_{50} = 2.6 μM, IN: IC_{50} = 41 μM, EC_{50} = 0.21 μM
60 R = Et, RT: IC_{50} = 1.1 μM, IN: IC_{50} = 51 μM, EC_{50} = 1.5 μM
61 R = $(CH_2)_2OH$, RT: IC_{50} = 3.7 mM, IN: IC_{50} = 19 μM, EC_{50} = 2.2 μM

Fig. (5). Complexing motif for *β*-diketoacid, the general scheme of the two metal chelating state for diketoacid-type compounds, and the possible complexing motif for scaffold **II** [62].



62

Sechi research group designed a scaffold **II** (Fig. (**5**)) with hydroxyl, carboxylic acid, and oxo functional groups, which could facilitate chelation of two metal ions in the active site of IN with a similar way as diketo acids or diketo acid-type compounds [62]. Compound **62** showed a potency in inhibiting IN strand transfer catalytic activity similar to the diketo acid inhibitor L-731,988 (IC_{50} = 0.9 μM vs. 0.54 μM, for **62** and L-731,988, respectively). The docking results proved that compound **62** very suitably mapped onto the quinolone 3-carboxylic acid pharmacophore, which nearly had the same mechanism as GS-9137 (Fig. (**6**)) [63].

In our research work [64], 1,4-dihydro-4-oxo-1,5-napthyridine-3-carboxylic acid moiety was employed as the hydrophilic subunit, which fitted topologically the require-

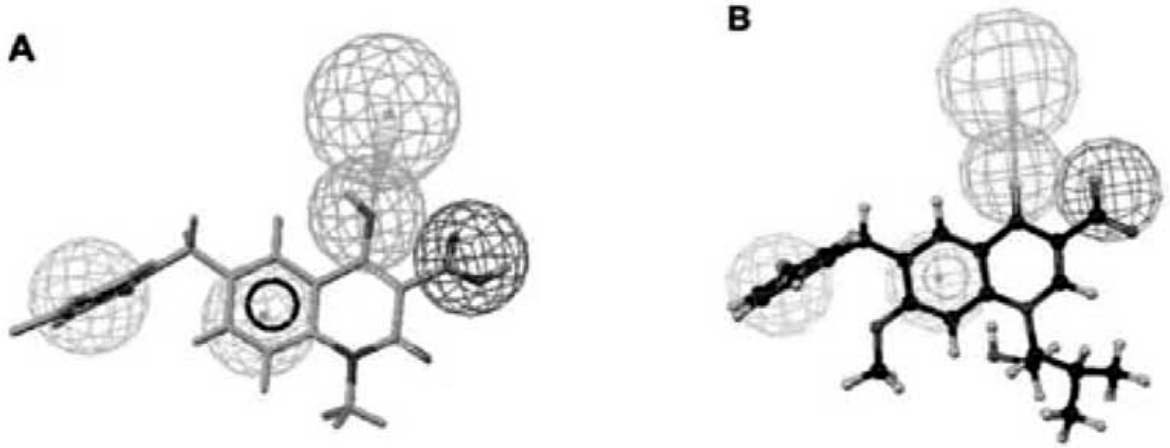


Fig. (6). A) Compounds **62** is mapped onto the quinolone 3-carboxylic acid pharmacophore [62]; **B**) The clinically studied HIV-1 integrase inhibitor GS-9137 is mapped onto the quinolone 3-carboxylic acid pharmacophore [63].

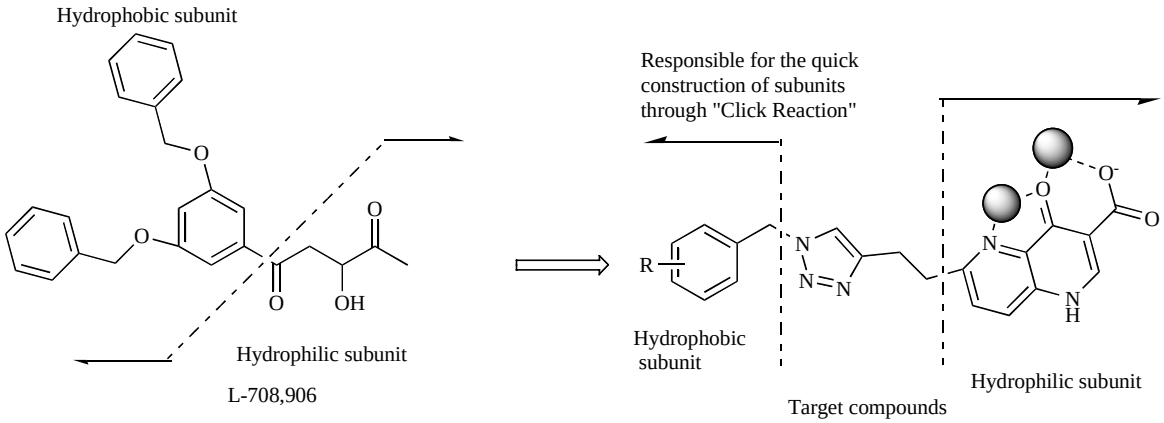


Fig. (7). Design of 1,4-dihydro-4-oxo-1,5-napthyridine-3-carboxylic acids bearing 1,2,3-triazole moiety [64].

ment to form a five-membered and a six-membered coordination systems with two metal co-factors (such as Mg^{2+}). Likewise, substituted benzene ring was used as a hydrophobic subunit (Fig. (**7**)). Unfortunately, anti-HIV IN activities of the desired compounds was not observed.

CONCLUSION

This review gives a brief summary of the recent 5-year achievements in discovering anti-HIV integrase agents of quinolone acid derivatives. Remarkable progress has been made since HIV integrase was recognized as a therapeutic target for treating HIV infection and preventing AIDS. Novel HIV inhibitors are needed to circumvent viral drug resistance or provide affordable and well-tolerated therapies. Quinolone acid derivatives played an important role as one of the kinds of HIV integrase inhibitors. GS-9137 (elvitegravir), which most probably became the next candidate of integrase inhibitor, was in the process of enrolling patients in the phase III clinical trials. It will be a powerful competitor with raltegravir. The latest technological advances (e.g., cheminformatics & bioinformatics), and an explosion in the optimization of lead compounds are together opening a new chapter of anti-HIV integrase inhibitors design. In addition, our laboratory should continue to develop novel agents with different mechanisms and drug targets, in order to prevent multidrug resistance to current antiviral drugs.

ACKNOWLEDGEMENTS

The authors thank Chinese Natural Science Foundation project (No. 30670497), National Key Basic Research Program of China (No. 2009CB930200), Beijing Natural Science Foundation (No. 7082006 and 7102009).

ABBREVIATIONS

AIDS = Acquired immunodeficiency syndrome

CC_{50} = Half maximal cytotoxic concentration

CCD = Catalytic core domain

CCR5 = Chemokine receptor 5

CRI = Co-receptor inhibitor

CTD = Carboxyl-terminal domain

DDE = Three acidic catalytic residues of D64, D116 and E152

DKA = Diketo acid

EC_{50} = Half maximal effective concentration

FDA = Food and Drug Administration

FI = Fusion inhibitor

HAART = Highly active antiretroviral therapy

HIV-1 = Human immunodeficiency virus type 1

IC_{50} = Half maximal inhibitory concentration

IN = Integrase

INI = Integrase inhibitor

NNRTIs = Non-nucleoside reverse transcriptase inhibitors

NRTIs = Nucleoside reverse transcriptase inhibitors

NTD = Amino-terminal domain

PIs = Protease inhibitors

QSAR = *Quantitative structure-activity relationship*

RT = Reverse transcriptase

SAR = Structure-activity relationship

ST = Strand transfer reaction

3'-P = 3′-processing

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Received: April 24, 2010 Revised: July 12, 2010 Accepted: July 16, 2010